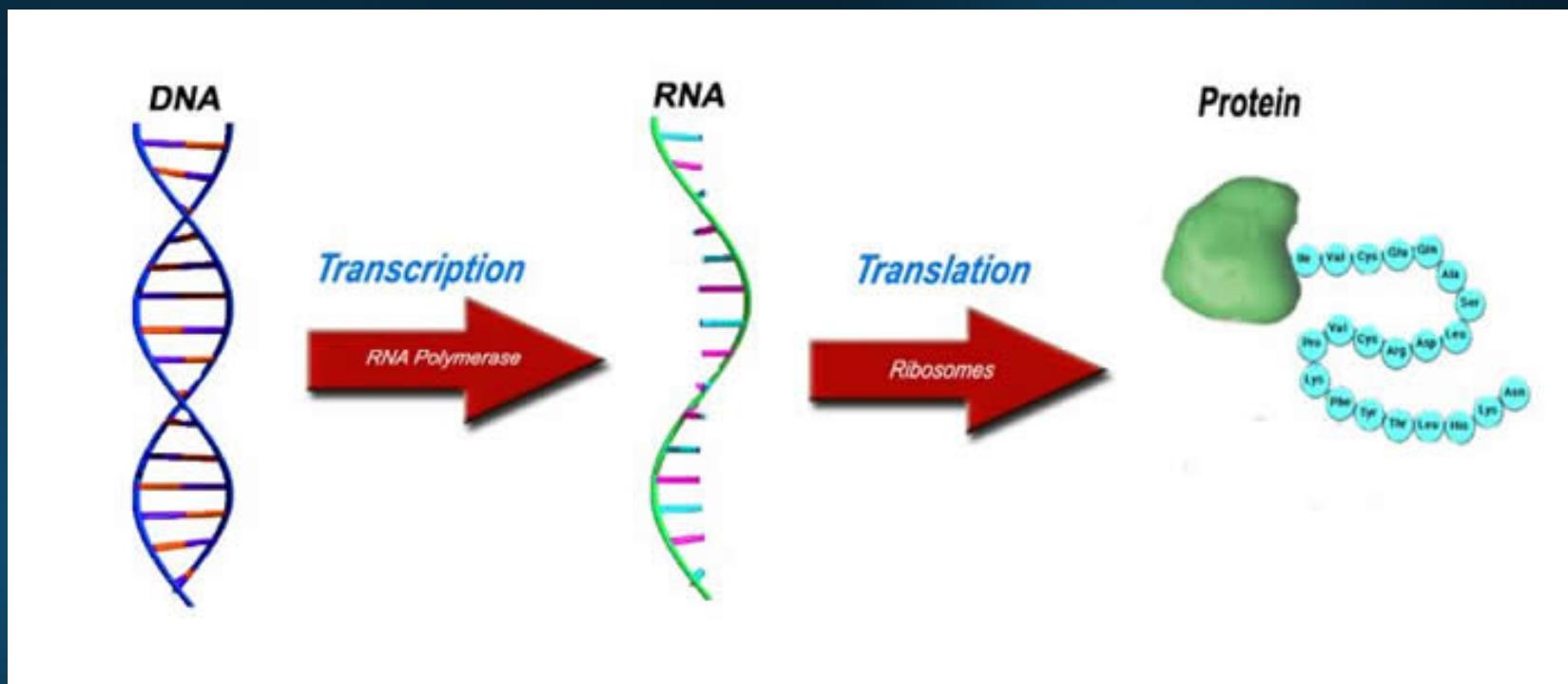


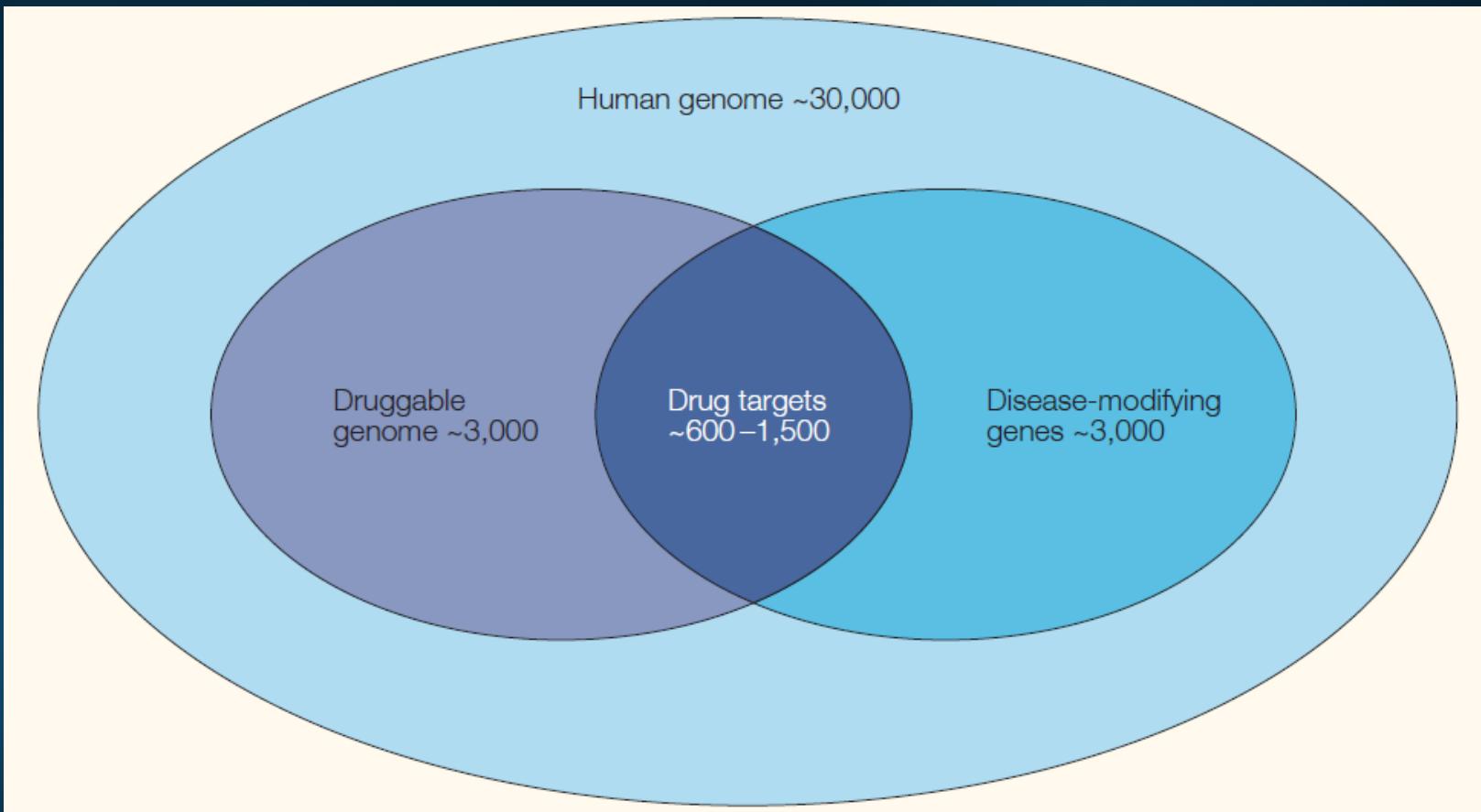
# Introduction to Gene Therapy

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# The central dogma of molecular biology



# The Druggable Genome



# ENCODE

- Less than 2% of the genome codes protein.
- More than 70% is transcribed.



# Too confined druggability?!!

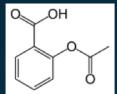




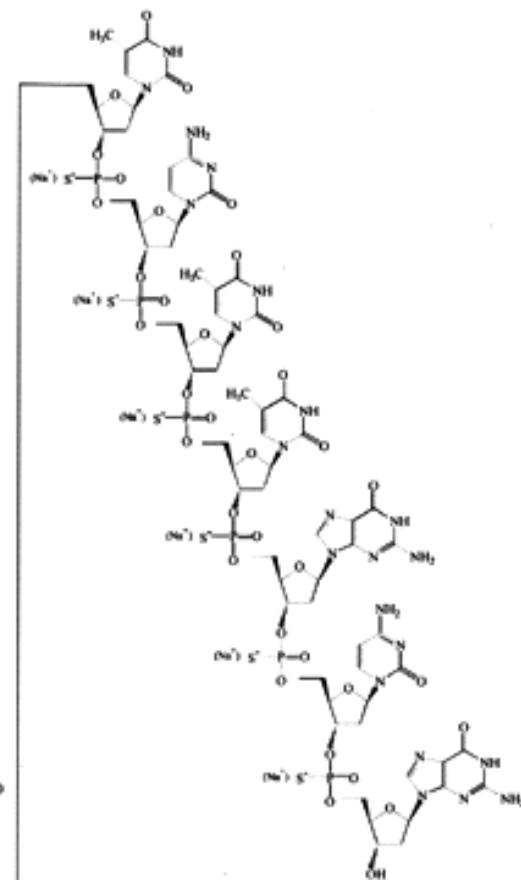
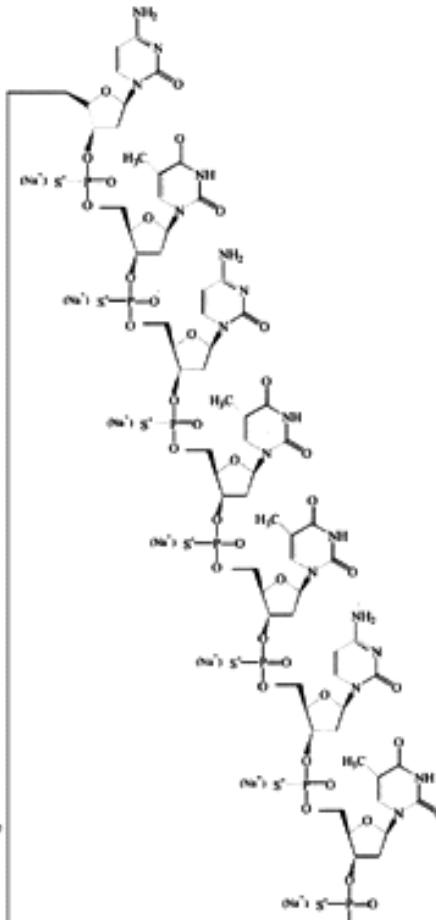
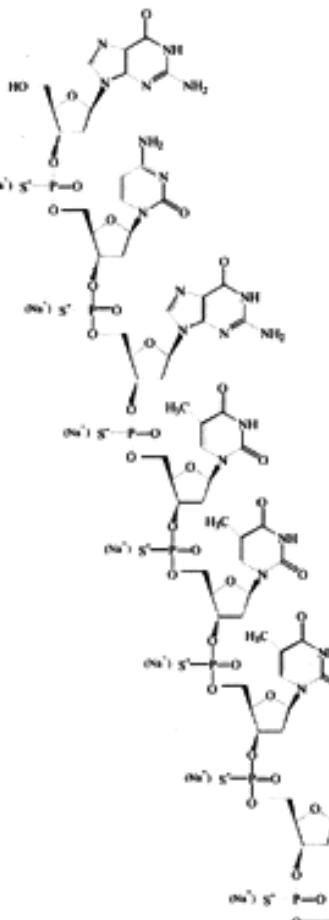
# Gene Therapy

1. Restoration of lost gene function.
2. Silencing of disease-causing genes.
3. Modification of gene function.

# Too big to get in...



Aspirin



Oligonucleotide

# How can we get it in?

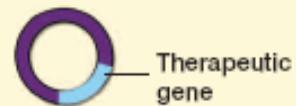


# Gene Therapy Vectors

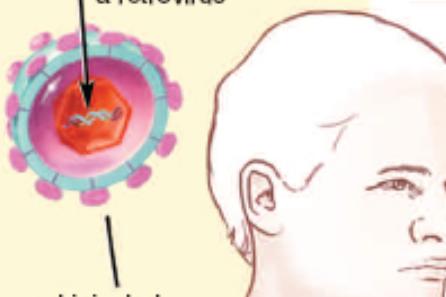


- Viral
- Non-viral

## Direct Delivery



The therapeutic gene is packaged into a delivery vehicle such as a retrovirus



...and injected into the patient

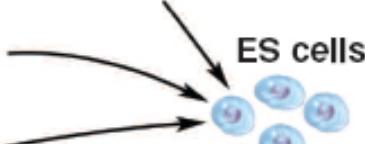
Target organ  
(e.g. liver)

## Cell-based Delivery

Genetically modified ES cells  
(can block immune rejection  
from patient)

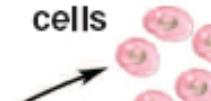
OR  
ES cell  
HLA bank

OR  
SCNT



Adult stem cells are  
isolated and propagated  
in the laboratory.

Adult stem  
cells

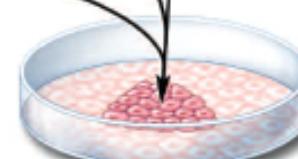


in vitro  
differentiated  
stem cell

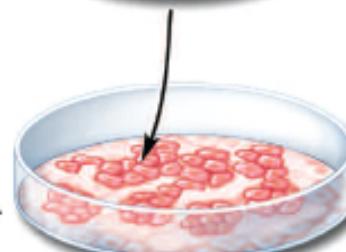


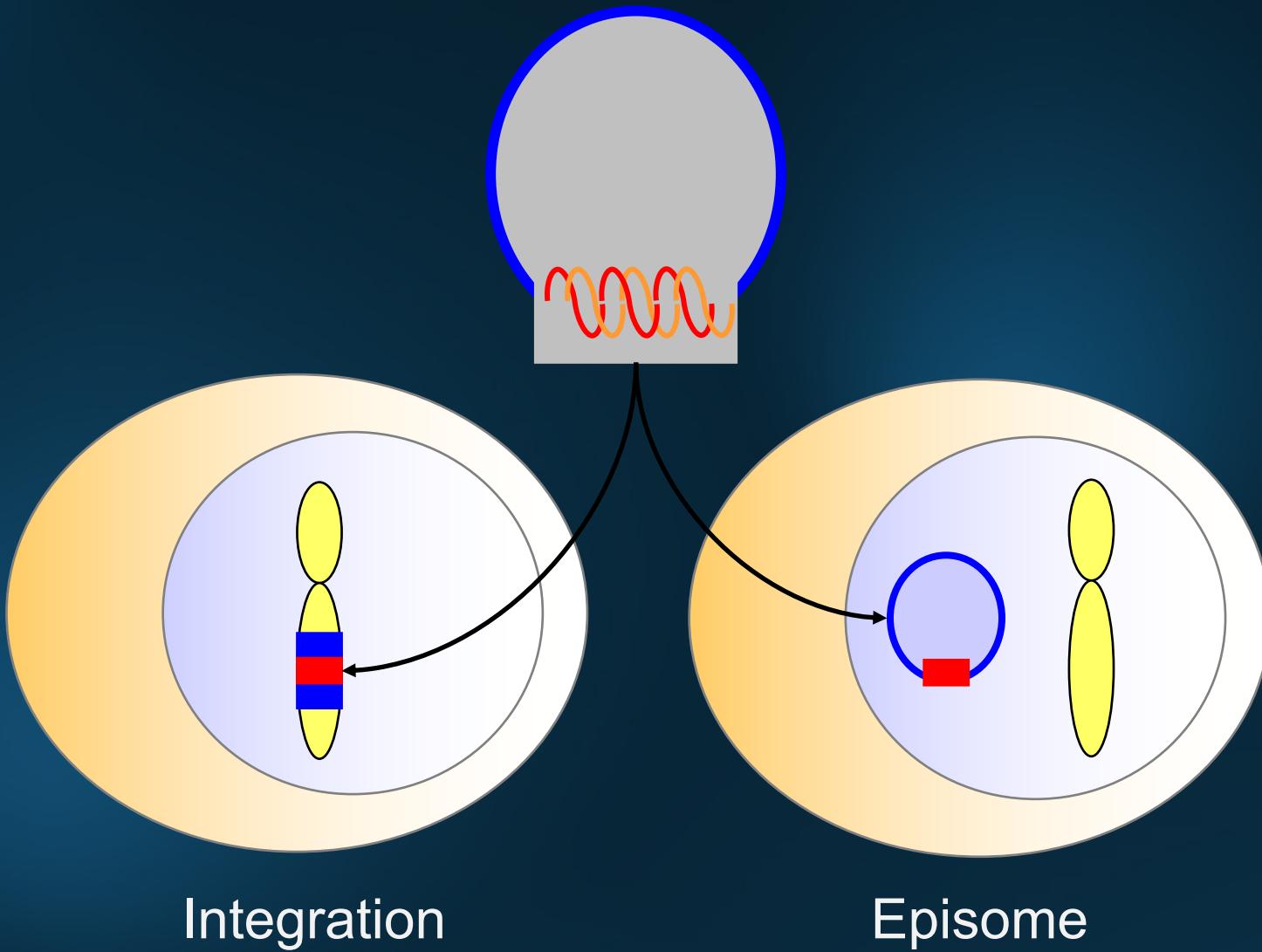
Therapeutic  
gene

The therapeutic gene is packaged into a  
delivery vehicle such as a retrovirus and  
introduced into the cells.



The genetically modified  
cells are reintroduced  
into the patient.





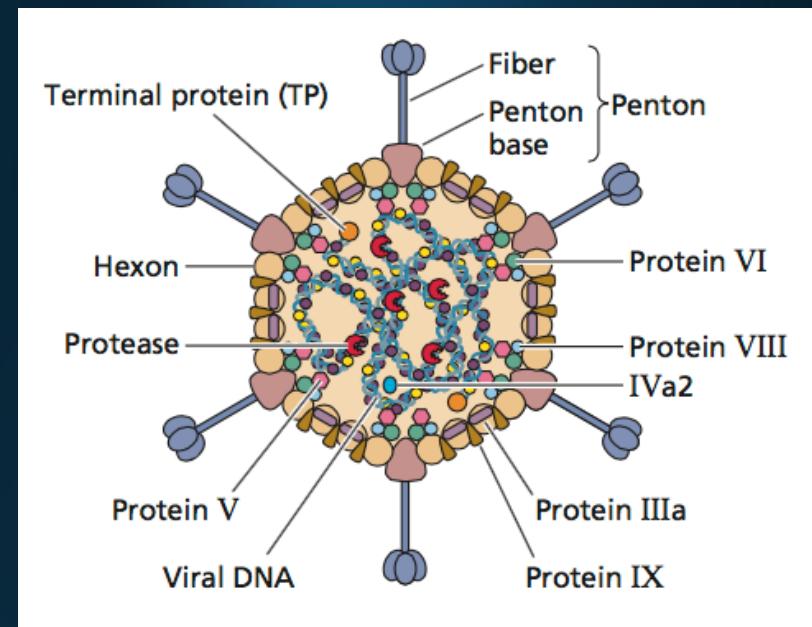


# Viral vectors

- Adenovirus vectors
- Adenovirus-associated virus vectors
- Retrovirus vectors

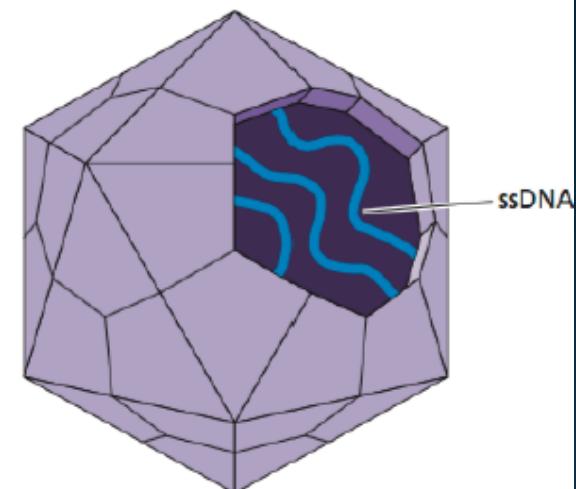
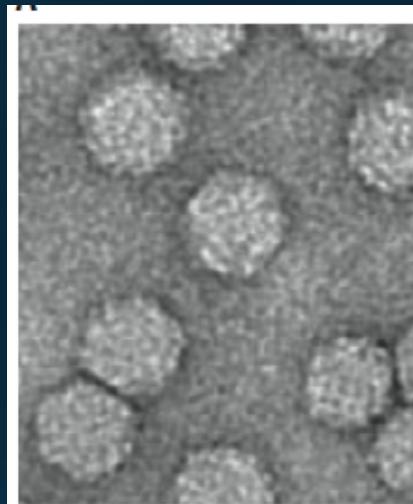
# Adenoviral vectors

- Efficiently infect post-mitotic cells
- Fast (48 h) onset of gene expression
- Episomal, minimal risk of insertion mutagenesis
- Up to 37 kb capacity
- Pure, concentrated preps routine
- >50 serotypes
- Drawback: immunity



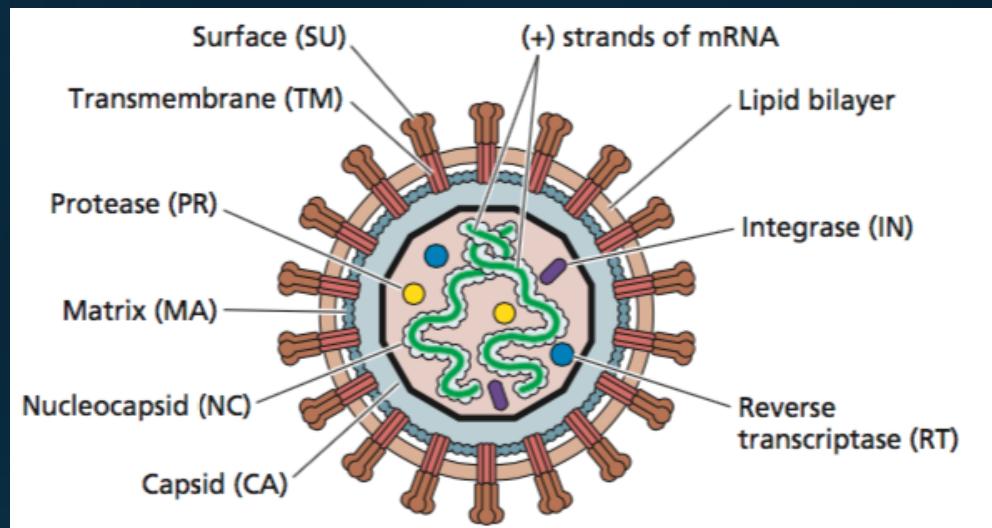
# Adeno-associated viral (AAV) vectors

- Human parvovirus
- Insert size 4.5 kb
- Long-term expression
- Broad host range
- Cell division requirement
- Non-pathogenic
- Drawback: immunity

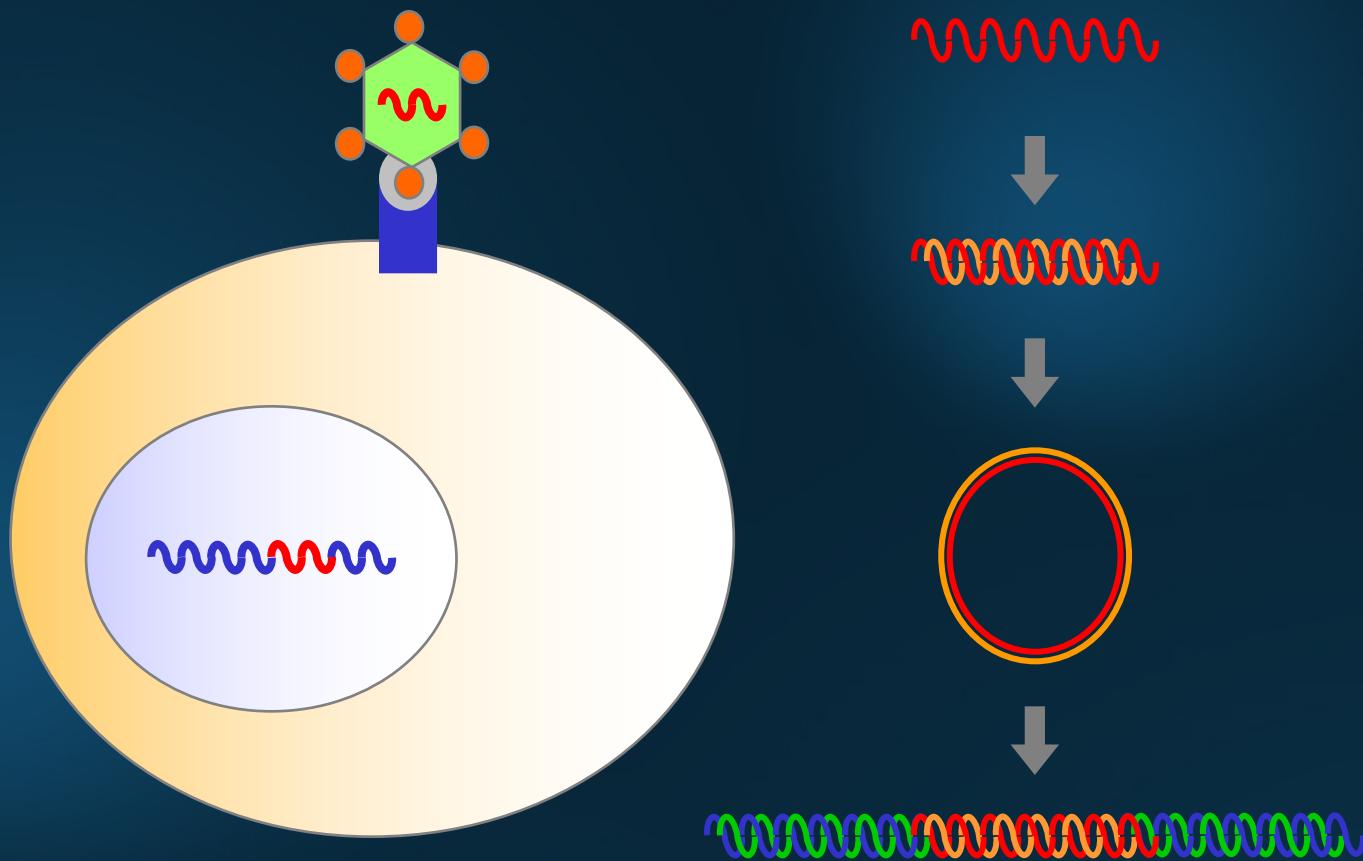


# Retroviral vectors

- Based on lentiviruses (HIV-1) or other retroviruses
- Insert size 7-8 kb
- Integration only dividing cells
- Short or long-term expression
- Broad host range
- Drawback: possibility for insertional mutagenesis



# Retroviral gene transfer





# Bumpy road early on..

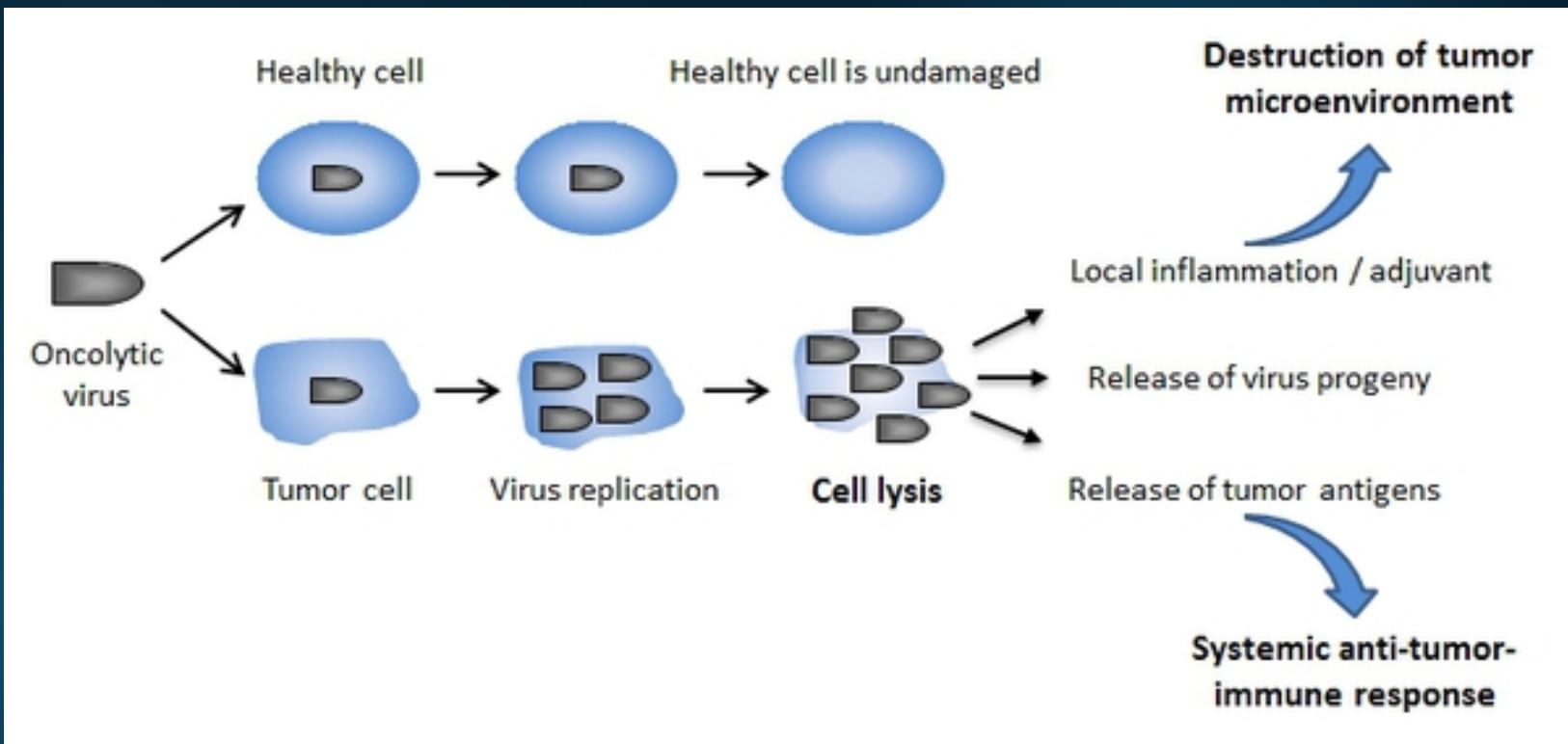
- Inflammatory responses → death of a patient enrolled in an experimentation for the deficit of ornithine-transcarbamylase using adenoviral vector in 1999.
- Insertional mutagenesis → development of leukemia in two patients with SCID-X1 treated with a retroviral vector in 2002.

- Leber's congenital amaurosis (LCA): AAV gene therapy (gene called *RPE65* involved in rhodopsin synthesis in the retina).
- Clinical trial in 2008 showed that four of six young adults who received the gene therapy could later sense more light and perform better in an obstacle course.



*Science, AAAS*

# Oncolytic Viruses



<http://www.viratherapeutics.com/technology/oncolytic-viruses/>

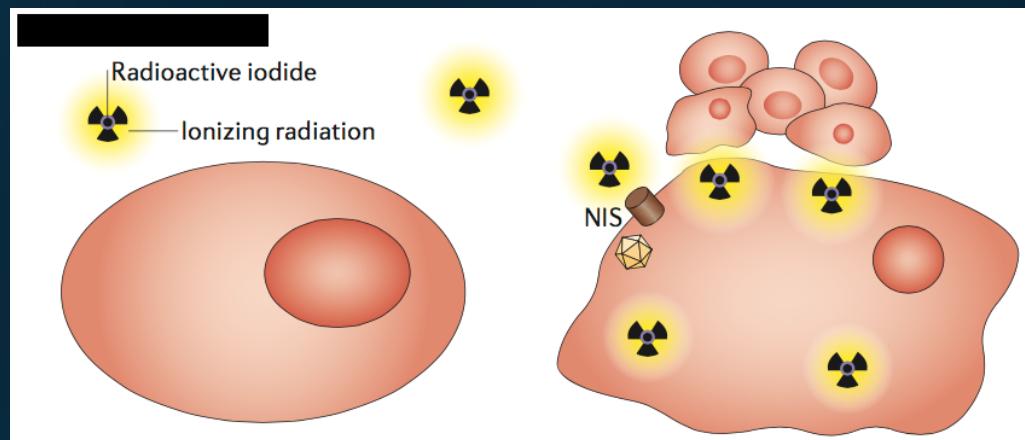
# Oncolytic Viruses



I am (not) a legend!

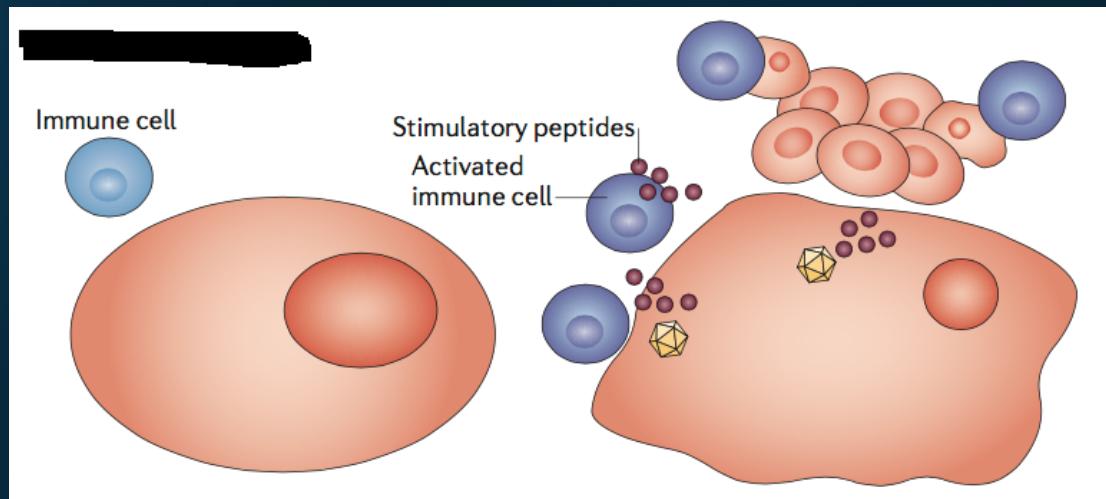
# Measles virus

- Attenuated vaccine strain
- Specific replication and preferential killing of tumor cells due to high CD46 receptor expression.
- Engineered to express sodium-iodide symporter (NIS)
  - +  $\gamma$ -emitting isotopes → specific imaging
  - +  $\beta$ -emitting isotopes → specific killing



# Granulocyte colony-stimulating factor GM-CSF

- Stimulate production of granulocytes and macrophages which stimulate adaptive immunity against tumor antigens.
- Herpes virus and adenovirus





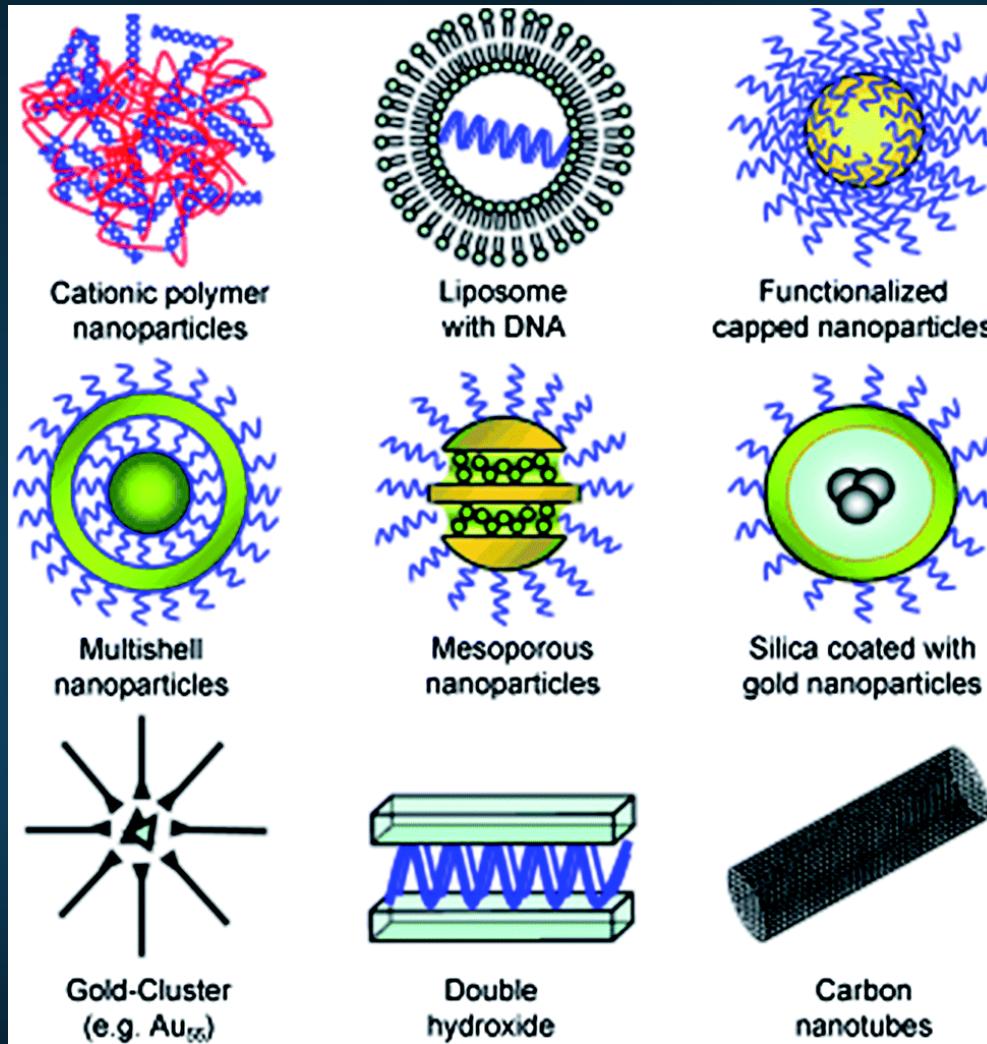
# Kymriah: First FDA approved oncolytic viral gene therapy

- [https://www.youtube.com/watch?v=mXDrg\\_ckhl&t=5s](https://www.youtube.com/watch?v=mXDrg_ckhl&t=5s)

# Non-viral gene therapy



# Virus mimicking vectors





# Advantages

- Versatile
- Safer
- Simpler

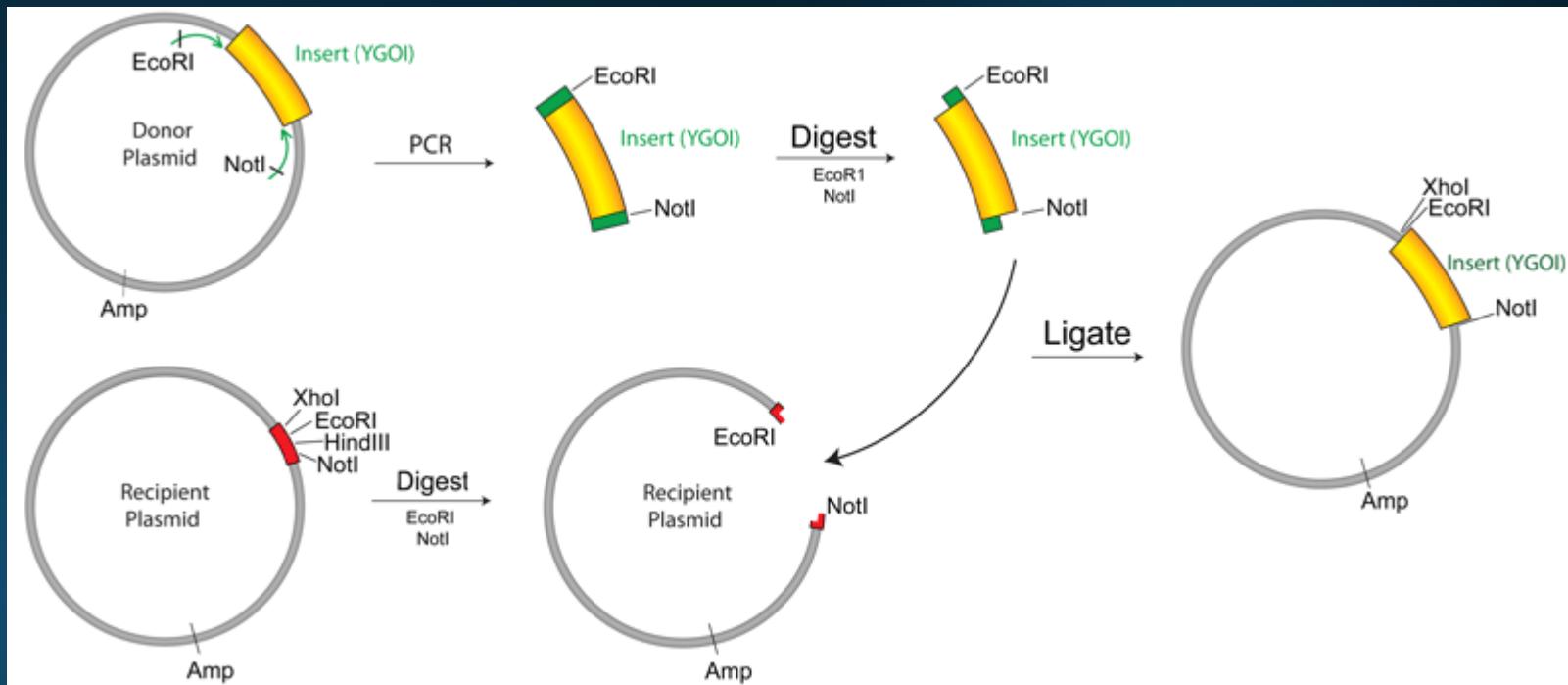
# Limitations

- Not as active as viruses.
- Low selectivity
- Poor biodistribution

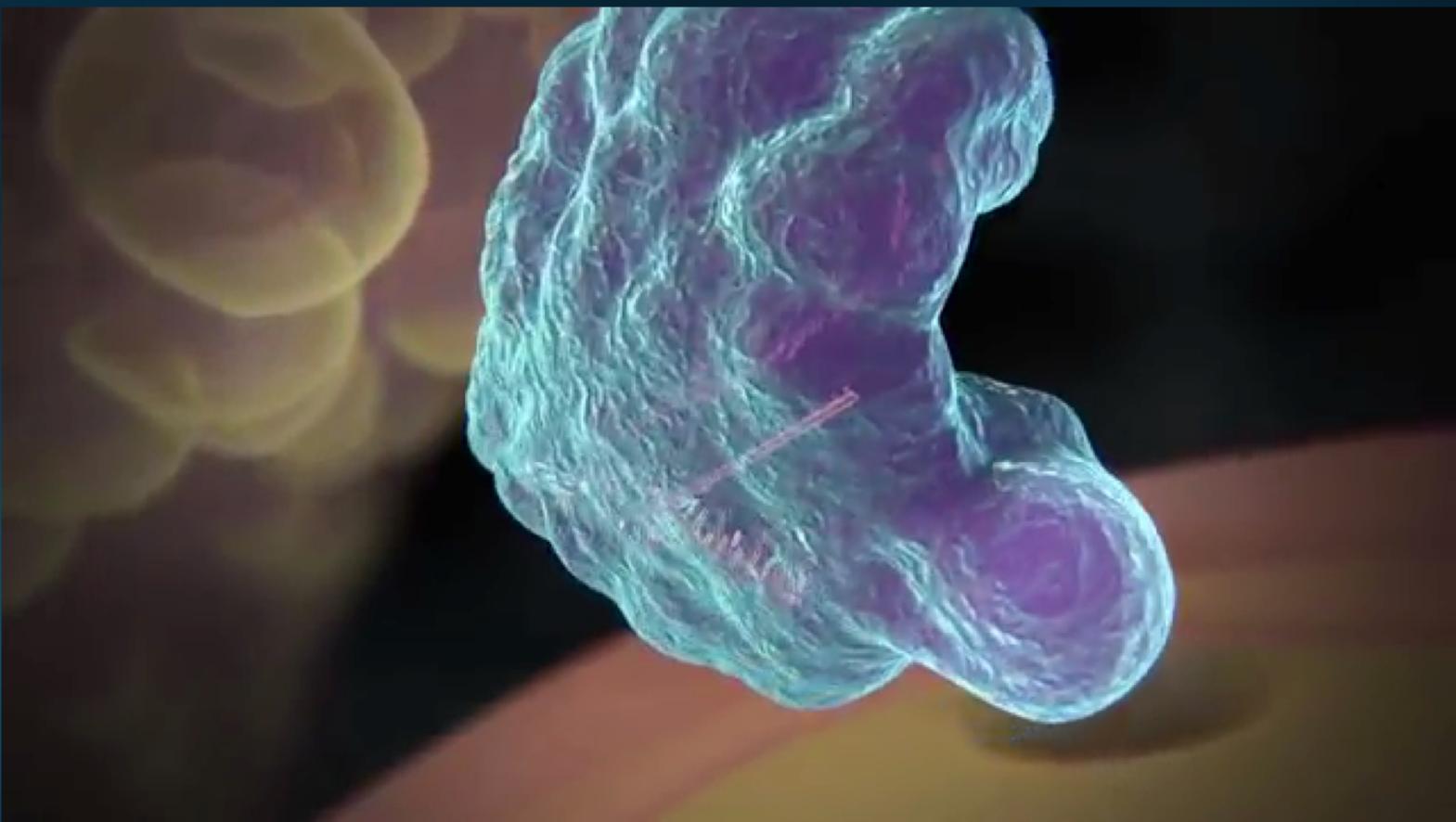
# Cargos



# Plasmid and mRNA delivery



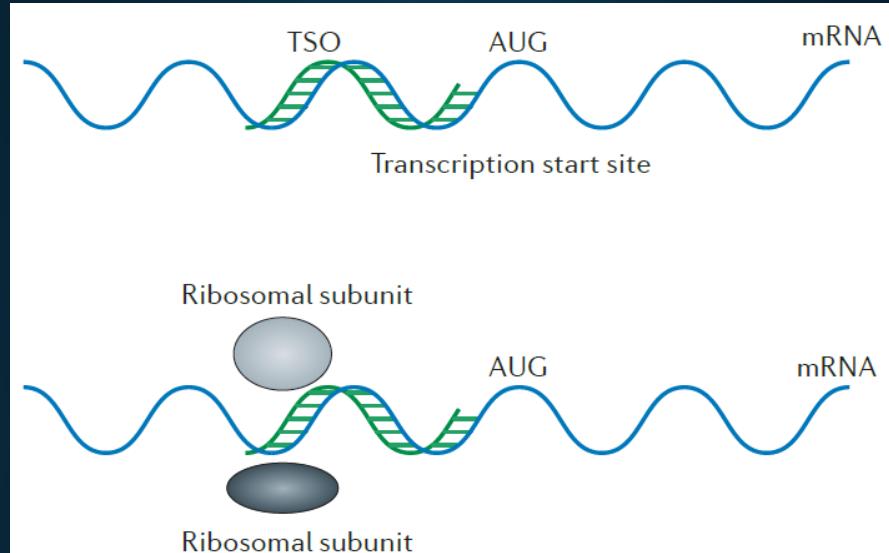
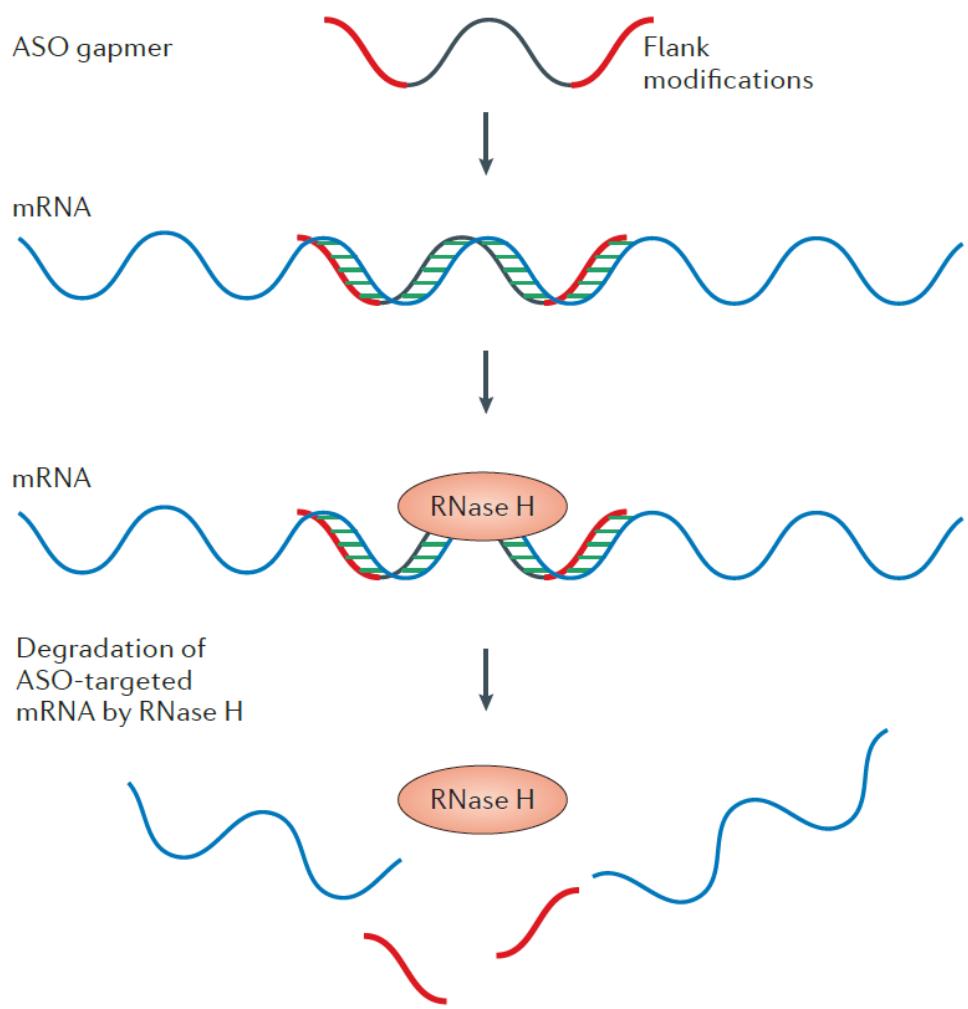
# Gene editing with CRIPR Cas9



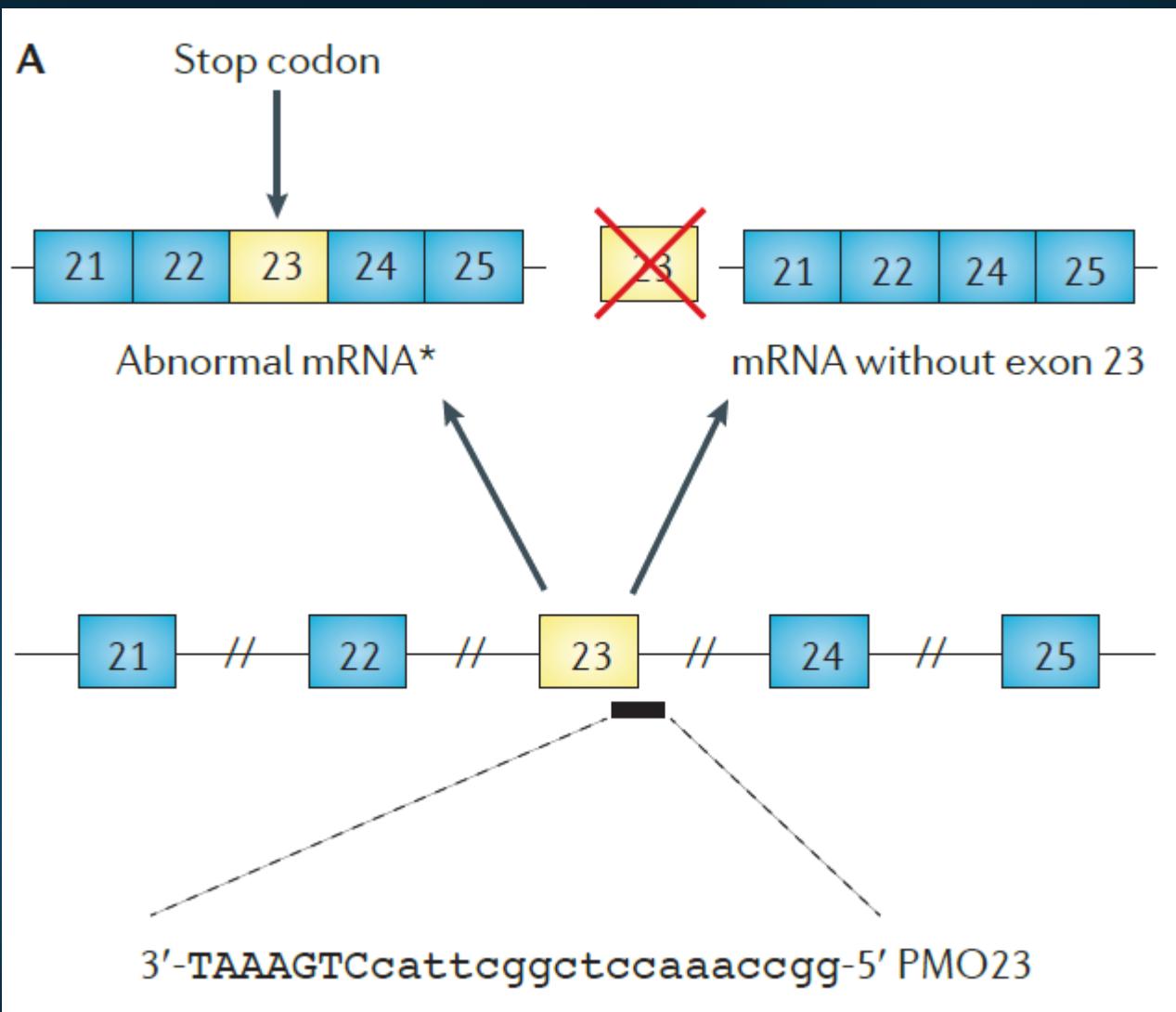
# Oligonucleotide gene therapy



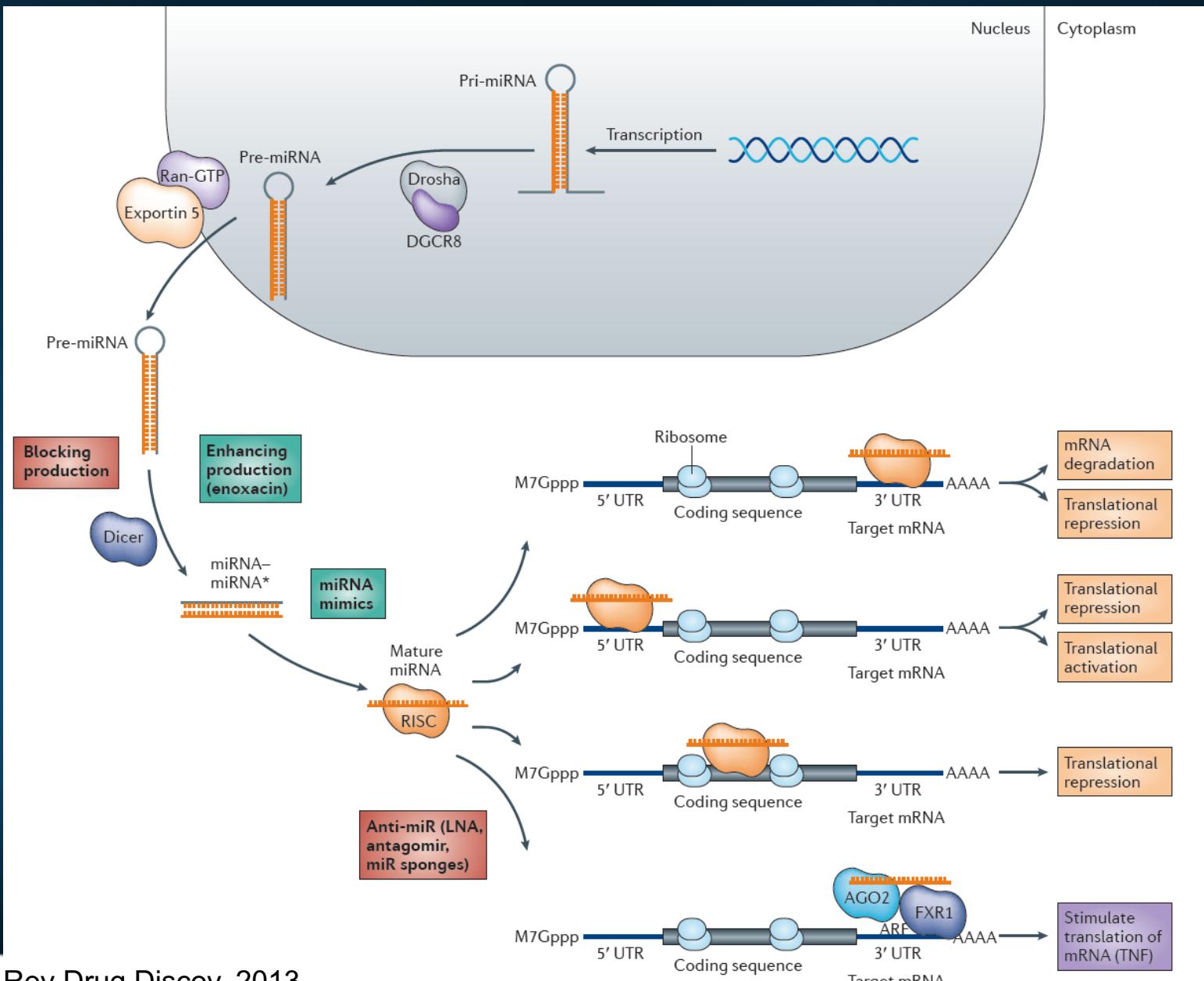
# Antisense



# Splice Switching



# Anti-miRNA





# Our work

- Peptide based gene delivery vectors.
- Oligonucleotide chemistry.
- The interface between nanotechnology and nanobiology.



# Oligonucleotide therapy for muscular dystrophy

- <http://www.nature.com/nm/journal/v21/n3/full/nm.3765.html?foxtrotcallback=true>

Thank you!

